In the Specification

Please replace the paragraph at page 2, lines 15 through page 3, lines 17 with the following paragraph:

The current invention concerns recently discovered intrinsic control site elements of constitutive nitric oxide synthases. These intrinsic control site elements, referred to as "regulatory peptides," include the regulatory peptide of endothelial nitric oxide synthase (ENOS), MSGPYNSSPRPEQHKSYKIRFNSVSCSDPLVSSWRRKRK ESSNTD (SEQ. ID. NO. 1); the regulatory peptide of neuronal nitric oxide synthase (NNOS) MRHPNSVQEERKSYKVRFNSVSSYSDSRKSSGDGPDLLRDNFE (SEQ. ID. NO. 2); a polypeptide specific to inducible nitric acid synthase (INOS), amino acids 600-615 of INOS (SEQ. ID. NO. 3). Based on this discovery, methods are now available to identify agents that modulate (activate or inhibit) NOS activity, as well as the agents themselves. Agents include agents that inhibit NOS activity by blocking calmodulin activation of the NOS enzyme; agents that inhibit NOS activity by blocking electron transfer from NADPH to an active site in NOS; agents that activate a constitutive NOS enzyme by antagonizing autoinhibition of a regulatory region of the NOS enzyme; and agents that modulate NOS activity by interacting with the regulatory peptide or spatially adjacent control regions. The agents include the peptides described above, as well as derivatives of these peptides, and homologous peptides. Homologous peptides include substantially isolated peptides having an array of at least two positively charged amino acids, and an amino acid sequence of at least about 60% homology, or about 67% homology, or about 80% homology, or about 90% homology, to the amino acid sequence of the ENOS regulatory peptide; peptides having an amino acid sequence of at least about 60% homology, or about 67% homology, or about 80% homology, or about 90% homology, to the amino acid sequence of the NNOS regulatory peptide; peptides having an amino acid sequence of at least about 60% homology, or about 67% homology, or about 80% homology, or about 90% homology, to the amino acid sequence of the INOS-specific peptide; and peptides having an amino acid sequence of at least about 60% homology, or about 67% homology, or about 80% homology, or about 90% homology, to the amino acid sequence of the negatively charged loops of the NOS enzymes. The invention further concerns nucleic acids

encoding the peptides, derivatives, and homologous peptides; fusions of peptides with proteins or other macromolecules (e.g., polysaccharides); peptidomimetics of the peptides, derivatives, and homologous peptides; and antibodies (either monoclonal or polyclonal antibodies, or fragments thereof) to the peptides, derivatives, and homologous peptides.

Please replace the paragraph at page 4, lines 9 through 23 with the following paragraph:

The current invention pertains to the discovery of the existence and identity of regulatory peptides of constitutive nitric oxide synthase (NOS) enzymes. As described in the Examples below, Applicant has identified the regulatory peptide of constitutive NOS enzymes as an intrinsic polypeptide insert in the flavin mononucleotide (FMN) binding domain of endothelial nitric oxide synthase (ENOS), MSGPYNSSPRPEQHKSYKIRFNSVSCSDPLVSSWRRKRKESSNTD (SEQ. ID. NO. 1) and in brain or neuronal nitric oxide synthase (NNOS) MRHPNSVQEERKSYKVRFNSVSSYSDSRKSSGDGPDLLRDNFE (SEQ. ID. NO. 2). Inducible nitric oxide synthase (INOS) lacks a similar polypeptide insert; instead, INOS has an INOS-specific region (the "INOS-specific polypeptide"), amino acids 600-615 of INOS (SEQ. ID. NO. 3), a short loop which is split and greatly extended by the introduction of the regulatory peptide (also referred to herein as the intrinsic peptide) in ENOS and NNOS. Applicant has also identified a core region of the ENOS binding domain: the array of positively charged amino acids, RRKRK (SEQ ID NO: 10), within the ENOS binding domain, alters activity of the enzyme.

Please replace the paragraph at page 20, lines 22 through 28 with the following paragraph:

These compounds can be manufactured by known methods. For example, a polyester corresponding to the peptide RRKRK (SEQ ID NO: 10) can be prepared by the substituting a hydroxyl group for each corresponding amine group on the R and K amino acids, thereby preparing a hydroxyacid and sequentially esterifying the hydroxyacids, optionally blocking the basic side chains and acids to minimize side reactions. Determining an appropriate chemical

A3

synthesis route can generally be readily identified upon determining the chemical structure using no more than routine skill.

Please replace the paragraph at page 29, lines 14 through 24 with the following paragraph:

In order to evaluate the functional significance of the putative inhibitory polypeptide, a series of synthetic polypeptides were designed which incorporated structural features of loop regions in the FMN domain. Polypeptides corresponding to promising recognition sites such as the RRKRK (SEQ ID NO: 10) motif were synthesized in lengths ranging from six to thirty five residues, as shown in Table 1, below. Polypeptides corresponding to both the ENOS and NNOS insertions were selected for evaluation. In addition to constructs based on the major insertions, polypeptides corresponding to the neighboring α>β loops in all three isoforms were synthesized, because of the possibility that these loops form a significant part of the binding site for the insertion in NOS. This possibility was suggested both by their proximity to the insertion and by their negative charge.

A4

Please replace Table 1 on page 30 with the following Table:

TABLE 1 Synthetic Peptides

Peptide	Der.[1]	Sequence	SEQ ID NO.
BO58-01	h ENOS	AVDTRLEELGGERT	35
BO58-02	NNOS	AVDTLLEELGGERT	22
BO58-03	m INOS	DIDQKLSHLGASQT	23
BO58-04	b ENOS	DDVVSLEHET	24
BO58-05	r NNOS	DIVHLEHES	25
BO58-06	m INOS	KASTLEEEQ	26
BO58-07	b ENOS	WRRKRK	12
BO58-08	b ENOS	SSWRRKRKESS	13

A5

BO58-09	h NNOS	QEERKSYKVRF	16
BO58-10	h NNOS	RPEQHKSYKIRF	17
BO58-11	r NNOS	SDSRKSSGDGPDLR	18
JX2	b ENOS	SSPRPEQHKSYKIRFNSVSCSDPLVSSWRRK RKESS	14
JX3	b ENOS	QHKSYKIRFNSVSCSDPLVSSWRRKRKE	15
JX4	h NNOS	QEERKSYKVRFNSVSSYSDSQKSSGDGPDL	19
PEP1		RPEQHKSYKIRF	27
PEP2		QEERKSYKVRFNSVSSYSDSRKSSGDGPDL	28

1 Derivation: h = human; m = mouse; b = bovine and r = rat.

Please replace the paragraph at page 32, lines 6 through 8 with the following paragraph:

The most effective inhibitory polypeptides contain the motif RRKRK (SEQ ID NO: 10) from the ENOS insertion. Partial inhibition of INOS could also be obtained with NNOS-based polypeptides.

Please replace the paragraph at page 33, lines 18 through 24 with the following paragraph:

The results of the experiments not only confirmed the function of the major FMN module insertion as the inhibitory polypeptide, but suggested a few details of the switching mechanism. While a number of the polypeptides could modulate CAM binding, the series containing the RRKRK (SEQ ID NO: 10) motif was the most instructive. The peptide with three residues following this motif (RRKRKESS) (amino acids 4-11 of SEQ ID NO:13) was a potent CAM antagonist with ENOS and NNOS. CAM binding was decreased almost to background levels, with effects seen at the 10 uM level.

Please replace the paragraphs at page 34, lines 1 through 28 with the following paragraphs:

A8

Polypeptides which terminated at the RRKRK (SEQ ID NO: 10) motif, including good inhibitors, were promoters of CAM binding. One polypeptide which had a single amino acid after this motif had no significant effect on CAM binding. Polypeptides based on the flanking loop regions had no significant effect on INOS activity, but tended to weakly promote the binding of CAM to constitutive NOS.

EXAMPLE 4 Mechanism of NOS Control

The results presented here provide powerful evidence that the major insertion in the FMN binding module is the inhibitory polypeptide of constitutive NOS, and that its absence in INOS accounts for the lack of sensitivity of INOS to calcium, and, in part, for its very tight binding of CAM. It appears that INOS has developed from an ancestral constitutive NOS-like protein by loss of the inhibitory peptide. The CAM binding site in INOS and constitutive NOS is apparently related to a similar basic region near the N terminal of P450 reductase, and may have developed from such a region in a common ancestral protein.

The inhibition of INOS by synthetic analogs of the constitutive NOS inhibitory polypeptides is related to the ability of the synthetic polypeptides to modulate CAM binding, but does not have the simple direct relationship expected if the mechanism of peptide inhibitor action was through CAM displacement. The reverse appears to be true: inhibition/activation of NOS at this site is driven by the occupancy of key sites by the inhibitory polypeptide, and CAM binding acts to modify the binding of the intrinsic inhibitory segment to a site or sites nearby on the surface of the enzyme.

It is not necessary to displace CAM in order to inhibit the enzyme at the control site. The data suggest that the binding domain of the inhibitory peptide has several regions. There is at least one recognition site which binds the RRKRK (SEQ ID NO: 10) motif, and there is indication of a second such site which recognizes sequences such as EERKSYKVRF (amino acids 2-11 of SEQ ID NO: 16) and EQHKSYKIRF (amino acids 3-12 of SEQ ID NO: 17) which occur in the N terminal half of the ENOS and NNOS insertions; peptides which lack RRKRK (SEQ ID NO: 10) but contain these sequences can be inhibitors and/or CAM binding modulators.

Please replace the paragraph at page 35, lines 1 through 11 with the following paragraph: